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**Physiological traits of *Penicillium glabrum* strain LCP 08.5568,
a filamentous fungus isolated from bottled aromatised mineral
water**

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Abstract

Penicillium glabrum is an ubiquitous fungus distributed world wide. This fungus is a frequent contaminant in the food manufacturing industry. Environmental factors such as temperature, water activity and pH have a great influence on fungal development. In this study, a strain of *P. glabrum* referenced to as LCP 08.5568, has been isolated from a bottle of aromatised mineral water. The effects of temperature, a_w and pH on radial growth rate were assessed on Czapeck Yeast Agar (CYA) medium. Models derived from the cardinal model with inflection (Rosso et al., 1993 An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. J Theor. Bio. 162, 447-463) were used to fit the experimental data and determine for each factor, the cardinal parameters (minimum, optimum and maximum). Precise characterisation of the growth conditions for such a fungal contaminant, has an evident interest to understand and to prevent spoilage of food products.

Keywords: *Penicillium glabrum*, predictive mycology, food spoilage, fungal growth, temperature, water activity, pH, cardinal values, mineral water

1. Introduction

Filamentous fungi are widely distributed in the environment and responsible for numerous spoilage of food products (Pitt and Hocking, 1997; Samson et al., 2004). In addition to the economic losses associated to their visual appearance, another concern is the possibility of off-flavours and mycotoxins production. The most widespread and frequent mould spoilages of food products are caused by several genera such as *Aspergillus*, *Fusarium* or *Penicillium*. Among this last genus, *Penicillium glabrum* is an ubiquitous and cosmopolitan fungus, frequently encountered in food manufacturing industry, due to its wide presence and its important conidiation (Pitt and Hocking, 1997). This filamentous fungus has been previously isolated in a large variety of products as cheese (Northolt et al., 1980; Hocking and Faedo, 1992), maize (Mislivec and Tuite, 1970), commercially marketed chestnuts (Overy et al., 2003), rice (Kurata et al., 1968), jam (Udagawa et al., 1977) and bottled water (Cabral and Fernandez Pinto, 2002; Ancasi et al., 2006). To our knowledge, this fungal contaminant does not seem to produce any known mycotoxin that could threaten the food safety and the consumer health (Pitt and Hocking, 1997). Nevertheless, no precise affirmation can be formulated due to inherent differences which could be observed among several strains of the same species. Despite its large implication in food contamination, to our knowledge, very few studies have been conducted to characterise precisely growth conditions of this species.

Growth of filamentous fungi is influenced by a variety of environmental or intrinsic factors. Temperature and water activity (a_w), for example, are recognised as the most important ones that determine the ability of moulds to grow (Dantigny et al., 2005). Other factors such as the composition and intrinsic factors of the product, especially pH, potentially influence the fungal development.

In order to analyse the physiological traits of a strain of *P. glabrum* isolated from a polyethylene terephthalate (PET) bottled aromatised mineral water, the present study aims at determining the cardinal values of this strain for temperature, a_w and pH. After investigating in solid

medium, its mycelial growth response towards different factors: temperature, a_w and pH, the development of this strain was studied by using a predictive mycology approach.

For over 20 years, predictive microbiology was focused mainly on food-pathogenic bacteria (Buchanan, 1993) and despite a similar interest, modelling filamentous fungal growth has not received the same level of attention. Actually, quantification of fungal growth is more complicated because, whereas bacteria reproduce by fission and grow homogeneously through a liquid medium, filamentous fungal growth implicated the development of tree-dimensional ramified hyphae with apical growth (Gibson et al., 1994; Gibson and Hocking, 1997). Taking account of these difficulties, the predictive mycology has been developed in several studies (Dantigny et al., 2005) by adapting different models used for bacterial investigations (Ratkowsky et al., 1983; Davey, 1989; Rosso et al., 1993; Baranyi et al., 1993; Miles et al., 1997). It appears that cardinal models with inflection (CMI) are suitable for modelling the effect of environmental factors on fungal growth (Rosso and Robinson, 2001). This kind of model originally developed for bacteria (Rosso et al., 1993; Rosso et al., 1995) has been successfully used to the effect of a_w on growth of several filamentous fungi such as *P. chrysogenum* or *Aspergillus flavus* (Sautour et al., 2001a).

In the present study, CMI were used to model the effects of temperature, a_w and pH on the radial growth rate of *P. glabrum*. This method allows the estimation of the cardinal values of this filamentous fungus for each tested factor. These results define the eco-physiological requirements of this fungal contaminant and has an evident interest to understand its contamination abilities in food manufacturing industry.

2. Materials and methods

2.1. Isolation and identification of the mould

Visible pellets were observed in a sealed PET bottle of aromatised mineral water. Three samples of 100 mL were shaken and filtered through sterile membrane porosity 0.45 μm (Millipore, Guyancourt, France). Visible hyphae were then transferred on Potato Dextrose Agar medium (PDA, Difco Laboratories, Detroit, MI, USA) and incubated for 7 days at 25 °C. A loopfull taken from a visible colony was examined under a microscope for morphological visualisation. Microscopic evaluation of the filamentous fungi isolated, indicated morphology similar to the description given by Pitt and Hocking for the genus *Penicillium* (phialides bearing chains of conidies) (Pitt and Hocking, 1997; Samson et al., 2004). The phialides were attached to the stipe directly, so the species produces monoverticillate penicilli and was classified in the subgenus *Aspergilloïdes*. Identification of the mould was further completed with inoculation of different media incubated at different temperatures following the reference method (Pitt, 1988). Observations were made on the morphology and diameters of the colonies and this filamentous fungus was characterised as *Penicillium glabrum* (Wehmer) Westling. This strain was registered as LMSA 1.01.421 in “Souchothèque de Bretagne” (University of Brest, France / www.ifremer.fr/souchotheque) and LCP 08.5568 in the fungal collection of Laboratory of cryptogamy, Museun Nationnal d’Histoire Naturelle (Paris, France / www.mnhn.fr).

2.2. Media preparation and culture conditions

The effect of each factor tested experimentally on the growth of this strain of *P. glabrum*, was studied in solid cultures using inoculum consisted in conidia harvested from 7 days-old grown in PDA medium at 25 °C, 0.99 a_w and pH 5.5. Conidia were suspended in 1 mL of sterile water with

136 0,01% Tween 80 (Sigma-Aldrich, Saint Louis, MO, USA). One drop of inoculum containing 10^4
137 spores /ml, was applied with thin pipette, on two points equidistant from the center and the edge of
138 Petri dish that contained the Czapeck Yeast Agar medium (CYA).

139

140 Temperature investigations: standard CYA medium was used and contained 3 % sucrose, 0.5
141 % yeast extract, 0.1 % K_2HPO_4 , 1.5 % agar and 1 % Czapek concentrate (5 % KCl, 30 % $NaNO_3$, 5
142 % $MgSO_4$, 7 H_2O , 0.01 % $FeSO_4$, 7 H_2O and 0.01 % $CuSO_4$, 7 H_2O). pH and a_w were respectively
143 measured at 6.8 and 0.99. After inoculation of 12 replicates (6 plates), for each condition tested,
144 media were then incubated for 7 days at temperatures in the ranges 5-45 °C.

145 Water activity investigations: CYA media were adjusted to various a_w from 0.79 to 0.99 by
146 substituting a part of water by glycerol (w/w) according to the relation of Langmuir (Lerici et al.,
147 1996): $M \text{ (water(g) / glycerol (g))} = 0.236 a_w / (1 - 0.99 a_w)$. Inoculations were realised, as described
148 previously except that inoculum was only applied in one point per plate. Triplicate plates were
149 inoculated for most a_w tested (0.79, 0.81, 0.83, 0.85, 0.87, 0.89, 0.91, 0.92, 0.93, 0.94) and for
150 highest values (0.95, 0.96, 0.97, 0.98 and 0.99), 8 replicated plates were realised. The different
151 media were incubated at 25 °C for 7 days. During the experiments, a_w of each medium was
152 stabilised by placing Petri dishes in 1,5 l closed boxes with a glycerol-water solution of the same a_w
153 as the medium (Sautour et al., 2001b). Stability of the different media was also controlled by
154 assessing a_w with FA-st/1 (CBX Scientific Instruments, Romans, France).

155 pH investigations: cultures of *P. glabrum* strain LCP 08.5568 were realised in different CYA
156 media with pH adjusted to each experimental condition. Precise volumes of sterile H_3PO_4 5M,
157 H_3PO_4 2M and NaOH 1M, were added respectively for pH 0.5-2.0, pH 3.0-7.0 and for pH 8.0-11.0
158 (Table 1). The adjusted media from pH 0.5 to 11.0 were inoculated as previously described using 8
159 replicates (4 plates) for each conditions tested. The different media were then incubated at 25 °C for
160 7 days. The pH values of each medium used, was also measured after 7 days of culture in order to
161 confirm their stability.

162

2.3. Growth rate calculation

Each factor was studied individually at 5 levels of temperature, 12 levels of a_w and 11 levels of pH containing for each level 12, 3 or 8 and 8 replicates respectively. The radius of the colony (mm) was measured in two directions at right angle and the mean was plotted against time (d). The radial growth rate μ (mm d^{-1}) was defined as the slope of the straight line.

2.4. Model equations

The relationship between the growth rate (μ) and the 3 environmental factors tested (temperature, a_w and pH) were assessed using the equations described below. The equations are based on the cardinal model with inflection (CMI) approach. For temperature the CMI originally developed by Rosso et al. (1993) was used

$$\mu(T) = \frac{\mu_{opt} (T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]} \quad (1)$$

The CMI modified by Sautour et al. (2001a) was used for a_w

$$\mu(a_w) = \frac{\mu_{opt} (a_w - 1)(a_w - a_{wmin})^2}{(a_{wopt} - a_{wmin})[(a_{wopt} - a_{wmin})(a_w - a_{wopt}) - (a_{wopt} - 1)(a_{wopt} + a_{wmin} - 2a_w)]} \quad (2)$$

For pH the CMI described by Rosso et al. (1995) was used

$$\mu(pH) = \frac{\mu_{opt} (pH - pH_{min})(pH - pH_{max})}{(pH - pH_{min})(pH - pH_{max}) - (pH - pH_{opt})^2} \quad (3)$$

2.5. Model fitting and determination of cardinal conditions

Before fitting, a square-root transformation was performed to homogenise the variance of the experimental growth rate (Dantigny and Bensoussan, 2008). Cardinal values were determined by iterative calculation based on minimising the sum of squares of the residual values (SSR) with NLINFIT function of MATLAB R2008A (The Math-works). 95 % confidence intervals were obtained by using traditional methods based on a linear approximation with NLPARCI function in MATLAB. For each factor modeled the Root Mean Square Error (RMSE) was calculated in order to measure the goodness of fit of each model. According to Ratkowsky (2004), this criterion should be preferred to the regression coefficient r^2 for non-linear models.

3. Results and discussion

3.1. Effect of temperature

The experimental growth results obtained in different conditions of temperature after 7 days of culture in CYA medium, were used to model the growth of this strain according to equation 1 of the CMI (Fig. 1). The minimal, optimal and maximal temperatures were estimated to 6.6, 24.3 and 33.8 °C respectively (Table 2). A good quality of fit was obtained as suggested by the low RMSE value of 0.077.

The optimal temperature around 24 °C for this strain of *P. glabrum*, is in accordance with literature data for this species that describes also an optimum around 25° C (Pitt and Hocking, 1997; Sinigaglia et al., 1998). Similar results were also reported in studies related to *P. chrysogenum* (Gonzalez et al., 1988), *P. expansum* (Lahlali et al., 2005), *P. digitatum* and *P. italicum* (Plaza et al., 2003). Meanwhile, optimal temperature varied slightly from 20 °C for *P. polonicum* (Nunez et al., 2000) to 30 °C for *P. citrinum* (Gonzalez et al., 1988; Montani et al., 1988). The range of

temperatures from 20 to 30 °C is frequently encountered in food manufacturing industries and may be also reached in non-refrigerated storage of some products as bottles of aromatised mineral water.

The maximal temperature condition for this filamentous fungus was close to 34 °C which is in accordance with some data reporting the absence of growth above 37 °C (Pitt and Hocking, 1997) but differs from others reporting a fungal growth up to 40 °C (Sinigaglia et al., 1998). Results obtained for this strain of *P. glabrum* also showed the minimal temperature condition of 7 °C which may differ from literature data, reporting a slight development of microcolonies up to 4 mm after several days at 5.0 °C (Pitt, 1988).

3.2. Effect of water activity

As reported previously (Sautour et al., 2001a), a gradual increase in the radial growth rate was exhibited at sub optimal water activities. In contrast a sharp decrease in the growth rate was observed was noticed between the optimum and 1 (Fig 2). The minimal and the optimal a_w were estimated to 0.820 and 0.983 respectively (Table 2). A good quality of fit was obtained as suggested by the low RMSE value of 0.078.

The minimal a_w for this stain 0.82 was less than the minimal value 0.88 a_w reported previously in another study for this species (Sinigaglia et al., 1998). Filamentous fungi are among the organisms capable of growing below 0.90 (Pitt and Hocking, 1997) and most *Penicillium* species presented a minimal a_w between 0.82 and 0.86 (Northolt et al., 1995). Similar a_w conditions are tolerated by some xerophilic *Penicillium* species as *P. chrysogenum* growing above 0.78-0.81 (Hocking and Pitt, 1979; Sautour et al., 2001b) or *P. roqueforti* growing from 0.82 (Gock et al., 2003). The minimal a_w for growth obtained in our study was lower than results obtained from *P. hordei*, *P. aurantiogriseum* (Marin et al., 1998) and *P. olsonii* (Lopez-Diaz et al., 2002). Several other *Penicillium* species showed minimal a_w around 0.90 as *P. expansum* (Lahlali et al., 2005), *P. verrucosum* (Cairns-Fuller et al., 2005) or *P. italicum* and *P. digitatum* (Lahlali et al., 2006).

The estimated optimal a_w condition was 0.98 which is in accordance with literature data on this species, reporting also the same value (Sinigaglia et al., 1998). Most *Penicillium* species also showed similar response to medium a_w and optimal conditions around 0.97-0.98 (Hocking and Pitt, 1979). For example, the optimal a_w for growth was estimated to 0.98 for *P. chrysogenum* using the same CMI than that described by eq (2) in this study (Sautour et al., 2001a).

3.3. Effect of pH

Radial growth rate was almost constant in the pH range 2.0-7.0 (Fig. 3). Experimental data were fitted by the model eq (3) rather satisfactorily, as suggested by the low RMSE value, 0.089 (Table 2). The optimal and the maximal pH values were 5.5 and 11.2 respectively but the minimal pH was estimated in the negative range at -2.1. Application of another model (Zwietering et al., 1992), gave with even a higher RMSE, aberrant minimal pH when applied to the same data (results not shown).

These results obtained showed the difficulty to model the growth response of this strain under very acidic conditions. Future studies should be directed to find a convenient model that fits correctly the pH growth response of this filamentous fungus. Nevertheless, the experimental data obtained gave some precious information as no fungal growth was observed at pH 0.5 which indicate that the minimal pH conditions seemed to be between 0.5 and 1.0. It differs from previous description of this species reporting a minimal pH value of 2.0 (Sinigaglia et al., 1998).

From the modeling of the pH response, the optimal pH condition of 5.5 and the large tolerance observed for this filamentous fungus towards a large range of pH conditions, were in accordance with literature describing optimal growth rate of many filamentous fungi around pH 5.0 (Pitt and Hocking, 1997) and in the pH range 3.0 to 8.0 (Wheeler et al., 1991). As reported in literature, sensibility of this strain of *P. glabrum* towards alkaline conditions appeared higher than

271 acidic ones. The pH response observed for this strain could be compared with other pH studies on
272 several *Penicillium* species conducted in solid medium (Wheeler et al., 1991). From these results, *P.*
273 *citreonigrum* seemed to present a similar response than *P. glabrum* and its optimum was defined at
274 pH 4.4-6.3. The results obtained in our study were also similar to those observed for *P. jensenii*
275 (Sacks et al., 1986) as this filamentous fungi seemed not very sensitive to pH range from 3.5 to 7.1
276 but showed an important fungal growth decrease just below at pH 3.3. *P. roqueforti* also showed a
277 large tolerance to several pH values tested from 4.5 to 7.5 (Gock et al., 2003). In a large range of
278 values, the medium pH seems to have a very low influence on the growth of this fungus as reported
279 also for several *Penicillium* species between pH 4.0-10.0 (Thompson et al., 1993). The tolerance
280 observed here for *P. glabrum* towards a large acid pH range may explain the presence of this
281 species on a large variety of food products of different pH. The pH sensibility increase in the
282 alkaline range until the estimated maximal pH value of 11.18. This value seemed coherent with the
283 results previously obtained on different *Penicillium* species (Wheeler et al., 1991).

284

285 Considering the good fit of the temperature and a_w models (RMSE of 0.077 and 0.078
286 respectively) and the estimated cardinal values, the method of CMI developed by Rosso *et al.*,
287 seemed well adapted to analyse the effect of both factors on the growth of this strain of *P. glabrum*.
288 The robustness of the approach of Rosso *et al.* of has been reported in a study on the effects of
289 temperature and a_w on *Aspergillus carbonarius* growth (Tassou et al., 2007). Analysis of the results
290 obtained with other predictive mycology methods, showed that Rosso *et al.* approach was the most
291 adapted to model the growth of this filamentous fungus in different conditions. This method has
292 been successfully used, for example, in *P. chrysogenum*, *Aspergillus flavus*, *A. parasiticus*, *A.*
293 *oryzae* to model the effect of a_w on fungal growth (Rosso and Robinson, 2001; Sautour et al.,
294 2001a). This method has also the advantage to define fungal growth rate (μ), by 4 parameters with
295 concrete physiological meaning: optimal growth (μ_{opt}) and minimal, optimal and maximal
296 conditions for each factor tested. Thus application and fitting of these models allowed to calculate

these parameters for each factor tested. For this reason, the use of CMI method has been well adapted to provide physiological characteristics of this strain of *P. glabrum* for temperature and a_w . Nevertheless some difficulties were shown to fit the experimental data with the CMI in very acidic conditions. Cardinal models are versatile tools that can adapt to the different shapes of the curves μ vs temperature and μ vs a_w . There are no reason that could prevent the CMI from fitting data pH vs pH with a good accuracy. The lack of fit that was demonstrated under acidic pH may be due to no data were available between pH 0.5 and 1, but this should be verified.

The different results obtained in this study provide useful background to improve characterisation of the strain of *P. glabrum* isolated from PET bottled aromatised mineral water. The microbiological quality of bottled mineral water is of great interest but has not been very largely investigated. In addition to indigenous bacteria that do not induce any risk to public health, mineral water may sometimes contain contaminants as bacteria or filamentous fungi. Some authors described that the fungal foreign bodies visible in the mineral water samples, were made up of pellets with a diameter of 3 to 20 mm (Fujikawa et al., 1997). The most frequent fungal genera isolated from mineral water were *Penicillium* followed by *Cladosporium*, *Trichoderma*, *Aspergillus*, *Alternaria* and *Acremonium* (Fujikawa et al., 1997; Liceaga-Gesualdo et al., 2001; Hageskal et al., 2006). Among the genus *Penicillium*, *P. citrinum* and *P. glabrum* were the 2 most isolated species (Cabral and Fernandez Pinto, 2002). Although filamentous fungi in water usually do not generate public health problems, nevertheless some of the fungi isolated from bottled mineral water as *Alternaria alternata* and *P. citrinum* have some toxigenic potential which could determine some health risk (Cabral and Fernandez Pinto, 2002).

The contamination of these products may be explained by microbial presence from the surrounding environment when filling and capping bottles of mineral water (Fujikawa et al., 1997). This last hypothesis was supplied by the fact that many filamentous fungi as some *Penicillium* species disperse a large number of spores in the environment.

323 In our study, the strain of *P. glabrum* isolated from aromatised mineral water, seemed to have
324 very low nutritional requirements as it can develop in visible pellets in such a poor nutritive
325 environment with slight carbohydrate concentrations, various salts and limited oxygen
326 concentration as only a small fraction of air is enclosed in tight sealed bottles. In literature, it was
327 also shown that sometimes, fungal contaminants could use as nutriment, organic compounds
328 releases during storage, from PET (Criado *et al.*, 2005), a beverage bottling material used for
329 conditioning a large variety of commercialised water as the one which is studied here. This
330 aromatised bottled mineral water presented a very high a_w , a pH at 7.0 and the storage of this
331 product was often made at room temperature (around 18-25 °C). The characteristics of this
332 aromatised mineral water may be favourable for the growth of this strain of *P. glabrum* by
333 extrapolating its physiological requirements obtained in solid medium. Several authors have
334 previously reported the presence of this species in commercialised water (Cabral and Fernandez
335 Pinto, 2002; Ancasi *et al.*, 2006). The contamination of this product by this filamentous fungus was
336 also explained by its ubiquitous presence in the environment and its large conidiation in the
337 atmosphere. Moreover, the physiological characteristics of this strain of *P. glabrum* seemed to
338 present important similarities with the temperature, a_w and pH requirements of another frequent
339 fungal contaminant of water products such as *P. citrinum* (Hocking and Pitt, 1979; Gonzalez *et al.*,
340 1988; Montani *et al.*, 1988; Wheeler *et al.*, 1991; Comerio *et al.*, 1998).

341
342 Precise characterisation of growth conditions of this strain of *P. glabrum* has an evident
343 interest to understand its contamination abilities in food manufacturing industry. The influence of
344 temperature, a_w and pH on fungal growth could be taken into account to maintain good conditions
345 on stored product. Nevertheless these results should be considered carefully as fungal
346 contamination of different products could also concern several other species than *P. glabrum* and
347 may interact in a competitive or associative way.

348

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350

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352

353 **References**

- 354 Ancasi, E.G., Carrillo, L., Benitez Ahrendts, M.R., 2006. Moulds and yeasts in bottled water and
355 soft drinks. Rev. Argent Microbiol. 38, 93-96.
- 356 Baranyi, J., Roberts, T.A., McClure, P., 1993. A non-autonomous differential equation to model
357 bacterial growth. Food Microbiol 10, 43-59.
- 358 Buchanan, R.L., 1993. Predictive food microbiology. Trends Food Sci Tech 4, 6-11.
- 359 Cabral, D., Fernandez Pinto, V.E., 2002. Fungal spoilage of bottled mineral water. Int. J. Food
360 Microbiol. 72, 73-76.
- 361 Cairns-Fuller, V., Aldred, D., Magan, N., 2005. Water, temperature and gas composition
362 interactions affect growth and ochratoxin A production by isolates of *Penicillium verrucosum*
363 on wheat grain. J. Appl. Microbiol. 99, 1215-1221.
- 364 Comerio, R., Fernandez, P., V, Vaamonde, G., 1998. Influence of water activity on *Penicillium*
365 *citrinum* growth and kinetics of citrinin accumulation in wheat. Int. J. Food Microbiol. 42,
366 219-223.
- 367 Criado, M.V., Fernandez, P., V, Badessari, A., Cabral, D., 2005. Conditions that regulate the
368 growth of moulds inoculated into bottled mineral water. Int. J Food Microbiol. 99, 343-349.
- 369 Dantigny, P., Bensoussan, M., 2008. The logarithmic transformation should be avoided for
370 stabilising the variance of mould growth rate. Int J Food Microbiol 121, 225-228.
- 371 Dantigny, P., Guilmart, A., Bensoussan, M., 2005. Basis of predictive mycology. Int. J. Food
372 Microbiol. 100, 187-196.
- 373 Davey, K.R., 1989. A predictive model for combined temperature and water activity on microbial
374 growth during the growth phase. J Appl Bacteriol. 67, 483-488.
- 375 Fujikawa, H., Wauke, T., Kusunoki, J., Takahashi, Y., Ohta, K., Itoh, T., 1997. Contamination of
376 microbial foreign bodies in bottled mineral water in Tokyo, Japan. J Appl Microbiol 82, 287-
377 291.
- 378 Gibson, A.M., Baranyi, J., Pitt, J.I., Eyles, M.J., Roberts, T.A., 1994. Predicting fungal growth: the
379 effect of water activity on *Aspergillus flavus* and related species. Int J Food Microbiol 23,
380 419-431.
- 381 Gibson, A.M., Hocking, A.D., 1997. Advances in the predictive modelling of fungal growth in
382 food. Trends Food Sci Tech 353-358.
- 383 Gock, M.A., Hocking, A.D., Pitt, J.I., Poulos, P.G., 2003. Influence of temperature, water activity

- 384 and pH on growth of some xerophilic fungi. Int. J. Food Microbiol. 81, 11-19.
- 385 Gonzalez, H.H., Resnik, S.L., Vaamonde, G., 1988. Influence of temperature on growth rate and lag
386 phase of fungi isolated from Argentine corn. Int. J. Food Microbiol. 6, 179-183.
- 387 Hageskal, G., Knutsen, A.K., Gaustad, P., de Hoog, G.S., Skaar, I., 2006. Diversity and significance
388 of mold species in Norwegian drinking water. Appl Environ. Microbiol 72, 7586-7593.
- 389 Hocking, A.D., Faedo, M., 1992. Fungi causing thread mould spoilage of vacuum packaged
390 Cheddar cheese during maturation. Int. J. Food Microbiol. 16, 123-130.
- 391 Hocking, A.D., Pitt, J.I., 1979. Water relations of some *Penicillium* species at 25 deg C. T Brit
392 mycol Soc 73, 141-145.
- 393 Kurata, H., Sakabe, F., Udagawa, S., Ichinoe, M., Suzuki, M., 1968. [A mycological examination
394 for the presence of mycotoxin-producers on the 1954-1967's stored rice grains]. Eisei
395 Shikenjo Hokoku 86, 183-188.
- 396 Lahlali, R., Serrhini, M.N., Friel, D., Jijakli, M.H., 2006. In vitro effects of water activity,
397 temperature and solutes on the growth rate of *P. italicum* Wehmer and *P. digitatum* Sacc. J.
398 Appl. Microbiol. 101, 628-636.
- 399 Lahlali, R., Serrhini, M.N., Jijakli, M.H., 2005. Studying and modelling the combined effect of
400 temperature and water activity on the growth rate of *P. expansum*. Int. J. Food Microbiol. 103,
401 315-322.
- 402 Lerici, C.R., Nicoli, M.C., Manzocco, L., 1996. Influenza dell'attività dell'acqua sulla tensione di
403 vapore dell'etanolo in sistemi modello alimentar. Industrie Alimentari 35, 13-17.
- 404 Liceaga-Gesualdo, A., Li-Chan, E.C.Y., Skura, B.J., 2001. Antimicrobial effect of lactoferrin digest
405 on spores of a *Penicillium* sp. isolated from bottled water. Food Res Int 34, 501-506.
- 406 Lopez-Diaz, T.M., Gonzalez, C.J., Moreno, B., Otero, A., 2002. Effect of temperature, water
407 activity, pH and some antimicrobials on the growth of *Penicillium olsonii* isolated from the
408 surface of Spanish fermented meat sausage. Food Microbiol 19, 1-7.
- 409 Marin, S., Sanchis, V., Saenz, R., Ramos, A.J., Vinas, I., Magan, N., 1998. Ecological determinants
410 for germination and growth of some *Aspergillus* and *Penicillium* spp. from maize grain. J.
411 Appl. Microbiol. 84, 25-36.
- 412 Miles, D.W., Ross, T., Olley, J., McMeekin, T.A., 1997. Development and evaluation of a
413 predictive model for the effect of temperature and water activity on the growth rate of *Vibrio*
414 *parahaemolyticus*. Int J Food Microbiol 38, 133-142.
- 415 Mislivec, P.B., Tuite, J., 1970. Species of *Penicillium* occurring in freshly-harvested and in stored
416 dent corn kernels. Mycologia. 62, 67-74.
- 417 Montani, M., Vaamonde, G., Resnik, S.L., Buera, P., 1988. Temperature influence on *Penicillium*
418 *citrinum* thom growth and citrinin accumulation kinetics. Int. J. Food Microbiol. 7, 115-122.
- 419 Northolt, M.D., Frisvad, J.C., Samson, R.A., 1995. Occurrence of food-borne fungi and factors for
420 growth. In: Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O. (Eds.), Introduction to
421 food borne fungi Centraalbureau voor Schimmelcultures, Baarn, pp. 243-250.

- 422 Northolt, M.D., van Egmond, H.P., Soentoro, P., Deijll, E., 1980. Fungal growth and the presence
423 of sterigmatocystin in hard cheese. J. Assoc. Off Anal. Chem. 63, 115-119.
- 424 Nunez, F., Diaz, M.C., Rodriguez, M., Aranda, E., Martin, A., Asensio, M.A., 2000. Effects of
425 substrate, water activity, and temperature on growth and verrucosidin production by
426 *Penicillium polonicum* isolated from dry-cured ham. J. Food Prot. 63, 231-236.
- 427 Overy, D.P., Seifert, K.A., Savard, M.A., Frisvad, J.C., 2003. Spoilage fungi and their mycotoxins
428 in commercially marketed chestnuts. Int J Food Microbiol 69-77.
- 429 Pitt, J.I., 1988. A laboratory guide to commun *Penicillium* species, Second edition. Food Science
430 Australia.
- 431 Pitt, J.I., Hocking, A.D., 1997. Fungi and food spoilage, Second edition. Blackie academic and
432 professional.
- 433 Plaza, P., Usall, J., Teixido, N., Vinas, I., 2003. Effect of water activity and temperature on
434 germination and growth of *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum*. J.
435 Appl. Microbiol. 94, 549-554.
- 436 Ratkowsky, D.A., 2004. Model fitting and uncertainty. In: McKellar, R.C., Lu, X. (Eds.), Modeling
437 microbial responses in food CRC Press, Boca Raton, Florida, USA, pp. 151-196.
- 438 Ratkowsky, D.A., Lowry, R.K., McMeekin, T.A., Stokes, A.N., Chandler, R.E., 1983. Model for
439 bacterial culture growth rate throughout the entire biokinetic temperature range. J Bacteriol.
440 154, 1222-1226.
- 441 Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient Model To Describe the
442 Combined Effects of Temperature and pH on Microbial Growth. Appl Environ. Microbiol 61,
443 610-616.
- 444 Rosso, L., Lobry, J.R., Flandrois, J.P., 1993. An unexpected correlation between cardinal
445 temperatures of microbial growth highlighted by a new model. J Theor. Biol. 162, 447-463.
- 446 Rosso, L., Robinson, T.P., 2001. A cardinal model to describe the effect of water activity on the
447 growth of moulds. Int J Food Microbiol 63, 265-273.
- 448 Sacks, L.E., King, A.D., Jr., Schade, J.E., 1986. A note of pH gradient plates for fungal growth
449 studies. J. Appl. Bacteriol. 61, 235-238.
- 450 Samson, R.A., Hoekstra, E.S., Frisvad, J.C., Filtenborg, O., 2004. Introduction to food and airborne
451 fungi, seventh edition. Centraalbureau voor Schimmelcultures (CBS), Utrecht, Netherlands.
- 452 Sautour, M., Dantigny, P., Divies, C., Bensoussan, M., 2001a. A temperature-type model for
453 describing the relationship between fungal growth and water activity. Int J Food Microbiol 67,
454 63-9.
- 455 Sautour, M., Rouget, A., Dantigny, P., Divies, C., Bensoussan, M., 2001b. Prediction of conidial
456 germination of *Penicillium chrysogenum* as influenced by temperature, water activity and pH.
457 Lett Appl Microbiol 32, 131-4.
- 458 Sinigaglia, M., Corbo, M.R., Ciccarone, C., 1998. Influence of temperature, pH and water activity
459 on "in vitro" inhibition of *Penicillium glabrum* (Wehmer) Westling by yeasts. Microbiol. Res.
460 153, 137-143.

461 Tassou, C.C., Panagou, E.Z., Natskoulis, P., Magan, N., 2007. Modelling the effect of temperature
462 and water activity on the growth of two ochratoxigenic strains of *Aspergillus carbonarius*
463 from Greek wine grapes. J Appl Microbiol 103, 2267-2276.

464 Thompson, D.P., Metevia, L., Vessel, T., 1993. Influence of pH alone and in combination with
465 phenolic antioxidants on growth and germination of mycotoxigenic species of *fusarium* and
466 *penicillium*. J Food prot 56, 134-138.

467 Udagawa, S.I., Kobatake, M., Kurata, H., 1977. [Re--estimation of preservation effectiveness of
468 potassium sorbate (food additive) in jams and marmalade (author's transl)]. Eisei Shikenjo
469 Hokoku 88-92.

470 Wheeler, K.A., Hurdman, B.F., Pitt, J.I., 1991. Influence of pH on the growth of some toxigenic
471 species of *Aspergillus*, *Penicillium* and *Fusarium*. Int. J. Food Microbiol. 12, 141-149.

472 Zwietering, M.H., Wijtzes, T., de Witt, J.C., van't Riet, K., 1992. A decision support system for
473 prediction of the microbial spoilage in foods. J Food prot 55, 973-979.
474
475